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# Antifungal medication is efficient in the treatment of sarcoidosis

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## Abstract:

**Objectives:** Fungi have been suspected of contributing to the pathogenesis of sarcoidosis. A previous intervention study demonstrated an improvement in the clinical condition in 15 out of 18 patients with a long-term history of sarcoidosis when antifungal medication was added to corticosteroids. The present study was performed to compare the effects of antifungal treatment with corticosteroid treatment in sarcoidosis.

**Methods:** Patients with newly diagnosed sarcoidosis were recruited. Corticosteroids were given to 39 subjects, corticosteroid + antifungal to 31, and antifungal only to 22 subjects. The effects of the treatments were evaluated at 6 months. X-ray scores were measured before and after treatment together with pulmonary diffusion capacity and two markers of sarcoidosis activity, that is, angiotensin-converting enzyme in serum (sACE) and chitotriosidase (CTO).

**Results:** X-ray scores as well as sACE and CTO decreased significantly in all groups. The X-ray score decreased slightly more among subjects in the groups that received antifungal medication compared with corticosteroids only ( $p < 0.001$ ).

**Conclusion:** The results suggest that antifungal treatment is as efficient as corticosteroid treatment against the granulomatous and inflammatory manifestations of sarcoidosis. This is probably because this treatment is directed towards the causative agent. Additional studies are required to define the phenotype, where the antifungal treatment was not efficient (4/22) and to perform long-term follow up to determine the risk of recurrence.

**Keywords:** antifungal, corticosteroids, fungi, sarcoidosis

## Introduction

Sarcoidosis is a multisystemic disease that is spread worldwide with an incidence that varies between countries and between ethnic groups and no causative agent(s) has been identified [Iannuzzi *et al.* 2007]. Research during recent years has increasingly suggested that sarcoidosis is related to fungal exposure. Several epidemiological studies have described a relation between sarcoidosis and environments with fungi or a high risk for fungal growth. Occupational risk factors were evaluated among 273 cases of sarcoidosis and 618 siblings without the disease [Kucera *et al.* 2003]. Specific risk exposures that imply possible fungal exposures were vegetable dusts, and indoor exposure to high humidity, water damage, or musty odours. In a case–control study on 706 newly recruited cases of sarcoidosis and equal numbers of age-, race-, and gender-matched controls, there were positive

associations between sarcoidosis and agricultural employment, and work environments with mould/mildew exposure [Newman *et al.* 2004]. An increased risk of sarcoidosis has also been found among firefighters [Prezant *et al.* 1999; Kern *et al.* 1993]. They work in an environment with high levels of aerosolized organic humidity laden materials. New cases of sarcoidosis were found among persons in an office building with mould problems [Laney *et al.* 2009].

If fungi were to contribute to the pathology in sarcoidosis, an intervention against fungi, either in the environment or in the patient, should result in an improvement of the disease. In a previous study, 18 patients with chronic sarcoidosis, unsuccessfully treated with corticosteroids, were given antifungal medication in addition to corticosteroids and 15 showed significant improvements after 6 months [Terčelj *et al.* 2007].

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In another study on four sarcoidosis patients, the dose of corticosteroids could be reduced after treatment with antifungal medication [Conron and Beynon, 2000].

To further evaluate if antifungal treatment is efficient in the treatment of sarcoidosis, a study was performed on patients with newly diagnosed disease. They were treated with corticosteroids, corticosteroid + antifungal medication, and antifungal medication only during a 6-month period.

## Material and methods

### Subjects

The clinic of respiratory diseases and allergy at the University Medical Centre, Ljubljana, Slovenia is one of the national centres for patients with sarcoidosis. For the diagnosis, the European Respiratory Society (ERS)/American Thoracic Society (ATS) criteria [ATS/ERS, 1999] were used. The routine at the clinic was to use bronchoscopy with 5–10 transbronchial biopsies of lung parenchyma and lymph nodes. Bronchoalveolar lavage was carried out with 200 ml to determine CD8+ cells and the CD4+/CD8+ ratio. The presence of noncaseating granulomas was verified histologically. If a biopsy was not considered representative, the patient underwent surgical pulmonary or lymph node biopsy. Aspiration was performed from one lobe for the culture of fungi and bacteria including tuberculosis. Most biopsies were stained (e.g. silver staining, Gomori) to identify the presence of fungal infection. IgA, IgM and IgG antibodies against *Candida* spp. and *Aspergillus* spp. were determined as well as the mannan antigen in blood.

For treatment, the procedure at the clinic was to observe the patients for 3–6 months to avoid treating cases with spontaneous regression. Indications for treatment were stages II and III with worsening of pulmonary symptoms, including cough and/or shortness of breath or chest pain, and one or more of the following criteria: deteriorating lung function with a fall in total lung capacity of 10% or more, a fall in forced expiratory volume in 1 s of 15% or more, a decrease in diffusion capacity (DL<sub>CO</sub>) of 20% or more, decreased gas exchange at rest or with exercise, progressive radiological changes or development of signs of pulmonary hypertension. All patients with extrapulmonary sarcoidosis were treated. Treatment was usually not given

to patients with stages II or III who were asymptomatic.

During the period January 2006 to June 2010, there were 182 patients at the clinic (24 stage I; 123 stage II, 55 treated; 34 stage III, 33 treated; 1 stage IV treated). Subjects for the study ( $n=92$ ) were recruited from among these patients on a voluntary basis. Extrapulmonary sarcoidosis was present in 29 of these and four were smokers and seven former smokers. None had any indications of fungal infection.

The study was approved by the Ethical Committee at the University Medical Centre, Ljubljana (198/05/04) and written informed consent was obtained.

### Treatments

The recruited subjects were alternately offered treatment with corticosteroid or corticosteroid + antifungal medication. Some patients refused corticosteroid treatment and were given antifungal medication only and *vice versa*. Antifungal medication was not given to those who had liver sarcoidosis or hepatic insufficiency. The treatments comprised corticosteroid (methylprednisolone 0.5 mg/kg ideal body weight every day during the first 4 weeks and then 0.25 mg/kg every second day), itraconazole (200 mg daily) or both medications together. The effect of the treatment was evaluated at 6 months.

The patients were also controlled after 2–3 months for side effects of the antifungal treatment. At that time, four out of the 22 subjects with antifungal treatment only had to be given corticosteroids because of worsening symptoms, lack of improvement in the parameters used to evaluate the clinical picture and symptoms or side effects of the antifungal treatment. These patients were excluded from the study.

### Clinical assessments

X-rays were taken before and after treatment. A grading scheme for the presence of granulomas was used as described previously [Terčelj *et al.* 2009, 2007]. The X-rays were read by two experienced radiologists, unaware of the status of the patient, grading granulomas according to a numerical score (0–4), judging the size and extension of the infiltrates (0 = normal, 1 = about 25% of lung field involved, 2 = up to 50%, 3 = up to 75%, and 4 = virtually the whole lung field involved). Repeat evaluations on two successive

occasions showed only minor deviations in the classification.

DL<sub>CO</sub> was measured using standard techniques. Inflammatory indicators of sarcoidosis were determined. Angiotensin-converting enzyme in serum (sACE) was determined using a colorimetric method and expressed as  $\mu\text{Kat/L}$  [Kasahara and Ashikara 1981]. Chitotriosidase (CTO) activity in serum was determined using 22  $\mu\text{M}$  4-methylumbelliferyl- $\beta$ -D-N,N',N''-triacetylchitotriose (Sigma) in citrate phosphate buffer (pH 5.2). The serum (5  $\mu\text{l}$ ) was incubated with 100  $\mu\text{l}$  substrate for 1 h at 37°C. The reaction was stopped with 2.5 ml of 0.3 M glycine/NaOH buffer (pH 10.6). Fluorescent 4-methylumbelliferone was read at excitation 365 nm and emission 465 nm using a Perkin-Elmer fluorimeter and the CTO activity was expressed in units of nmol/h/ml.

#### Statistical treatment

Differences between groups were evaluated using the Mann–Whitney *U*-test and Fisher's exact

test. The level of significance was set at 0.05, two-sided.

#### Results

The characteristics of the different treatment groups are shown in Table 1.

The three treatment groups were relatively homogenous except for the CD4+/CD8+ ratio, which was slightly higher in the group treated with antifungal medication only (not statistically significant).

The different endpoints before and after treatment are shown in Table 2.

The group treated with corticosteroid + antifungal medication had a slightly higher X-ray score before treatment compared with the group receiving corticosteroid only. After treatment the X-ray scores decreased significantly in all groups (all  $p < 0.001$ ). The decrease was larger in the group receiving antifungal medication and corticosteroid + antifungal medication,

**Table 1.** Basic characteristics of the different treatment groups.

Parameter	Corticosteroid	Antifungal treatment	Corticosteroid + antifungal treatment
<i>n</i>	39	18	31
Age, mean	46.7	41.3	43.6
% females	27	31	42
Stage II	28	11	16
Stage III	11	7	14
Stage IV	0	0	1
CD4/CD8	5.8 [0.5]	7.7 [2.0]	6.8 [0.8]
Mean and [standard error of the mean].			

**Table 2.** Different endpoints before and after treatment.

Treatment group	C	AF	C+AF
<i>N</i>	39	18	31
X-ray grade before	1.9 [0.1]	1.7 [0.1]	2.4 [0.2]
After	1.1 [0.2]	0.2 [0.1]	1.1 [0.1]
Change %	–32	–88	–54
DL <sub>CO</sub> % of predicted	84 [3.3]	91 [3.5]	87 [3.2]
After	87 [3.3]	97 [2.2]	93 [3.0]
Change %	4	7	7
sACE $\mu\text{Kat/L}$ before	0.40 [0.03]	0.45 [0.05]	0.44 [0.04]
After	0.24 [0.02]	0.35 [0.04]	0.25 [0.02]
Change %	–40	–22	–43
CTO nmol/h/mL before	806 [111]	441 [104]	768 [94]
After	340 [41]	205 [63]	271 [45]
Change %	–51	–51	–65
AF, antifungal treatment; C, corticosteroid; C+AF = corticosteroid + antifungal. Mean and [standard error of the mean].			

compared with the group receiving corticosteroids only (both  $p = 0.001$ ).

DL<sub>CO</sub> improved in the group receiving corticosteroids only ( $p = 0.08$ ). The same improvement was found in the group receiving antifungal medication. Regarding sACE, there was a significant decrease in the group receiving corticosteroids ( $p < 0.001$ ). In the group with antifungal medication, the decrease was also significant ( $p < 0.009$ ), although it was slightly lower compared with the group receiving corticosteroids (difference not significant). For CTO, the decrease after treatment was significant in all groups ( $p < 0.001$ ,  $= 0.010$  and  $< 0.001$ ). There was a slightly higher decrease in the group receiving corticosteroid + antifungal medication compared with the group receiving antifungal only ( $p = 0.025$ ).

Table 3 shows the distribution of X-ray score changes in the three groups after treatment. In comparison to those receiving corticosteroids only, the groups receiving antifungal medication had a lower number of patients who showed no change and a significantly larger number who improved 2 scores ( $p = 0.0002$ , Fisher's exact test). In the corticosteroid-treated group, 27 patients showed some improvement (69%) compared with all (100%) in the group receiving antifungal medication and 26 (84%) in the group receiving the combined treatment.

Conclusion

The major finding in the study was that treatment with antifungal medication was just as efficient against sarcoidosis as corticosteroid treatment. X-ray scores of granulomas improved and inflammatory markers of the disease decreased. In addition, the improvement in X-ray scores after treatment was greater in the groups receiving antifungal medication compared with the group receiving corticosteroids only.

The effects on other parameters of disease were similar to those of corticosteroids.

The study has some limitations. The evaluation of X-rays was made based on a score system where the extent of the granulomatous lesions was judged. This method has, however, been used in previous studies and the results were found to be related to the treatment [Terčelj *et al.* 2009, 2007]. In future studies other techniques such as positron emission tomography scan could be used to avoid subjective judgments [Keijsers *et al.* 2008]. In addition to X-ray scores the severity of the disease was estimated by measuring several parameters of inflammation. Previous reports have suggested that pulmonary function tests are sufficient to judge severity [Keir and Wells, 2010], but other reports suggest that determinations of CTO are closely related to X-ray scores and express the severity of the disease [Terčelj *et al.* 2009]. CTO is, however, not specific for sarcoidosis and high levels are also found in other inflammatory diseases.

The study was originally planned as a randomized administration of the antifungal medication and the corticosteroid treatment. This could not be accomplished as several patients refused antifungal medication, particularly because of the potential side effects. Others insisted on the antifungal treatment as they were aware of the results from the previous study [Terčelj *et al.* 2007]. There was thus a conflict between the scientific design and ethical considerations. For us the latter were the most important. From a purist point of view, the lack of a randomized design might be taken as a drawback but from clinical and scientific points of view, there is no reason to believe that this would have affected the outcomes as both X-ray readings and the analysis of inflammatory mediators were made without knowledge of the treatment.

Table 3. Changes in X-ray scores after treatment.

Parameter	Corticosteroid	Antifungal treatment	Corticosteroid + antifungal treatment
<i>n</i>	39	18	31
No change	12	0	5
Score 1	26	10	12
Score 2	1	7	14
Score 3	0	1	0
Mean and [standard error of the mean].			



A major difficulty in evaluating treatment schemes for sarcoidosis is the relatively high rate of spontaneous regression. In a previous antifungal intervention study, the patients had a disease history of more than 6 months and were chosen from those where corticosteroid treatment had little effect [Terčelj *et al.* 2007]. In that case, the influence of spontaneous regression was lower than in the present study. To decrease the risk for spontaneous regression, there was a 3–6 months expectancy period before the patient was recruited, which decreased but did not eliminate the risk for including cases with spontaneous regression. Based upon previous experience, it is highly unlikely that all the improvements seen in this study would be due to spontaneous regression or that there would be more cases of regression in the groups receiving antifungal medication.

There were no signs of fungal infection in the tissues examined nor were there any signs of mycosis. This implies that the underlying pathological mechanism is not dependent on viable fungi but rather the presence of specific agents in the fungal cell [Terčelj *et al.* 2008]. One such agent could be  $\beta$ -glucan, which is abundant in the fungal cell wall and has been shown to influence the immune system including the development of granuloma [Rylander, 2010]. Measures of fungi in the homes of patients with sarcoidosis have also demonstrated a significantly higher exposure than controls among those undergoing treatment or with a recurrence of the disease [Terčelj *et al.* 2011].

The results from this study confirm those from a previous pilot study where patients with long-term sarcoidosis were given antifungal treatment in addition to corticosteroids [Terčelj *et al.* 2007]. Corticosteroids are the standard treatment in sarcoidosis but it is agreed that it is symptom rather than cause oriented. Previously assessed alternatives to corticosteroid treatment include chloroquine [Silzbach and Teirstein, 1964], tetracyclines [Bachelez *et al.* 2001], and infliximab [Keijsers *et al.* 2008]. In contrast to these treatments, the concept of antifungal treatment is based on an environmental exposure concept as well as previous clinical observations of patients treated for fungal infections where a simultaneous improvement of sarcoidosis was observed [Terčelj *et al.* 2007]. In this way it is similar to an intervention study on severe asthma where antifungal medication was given based on

observations of fungal exposure [Denning *et al.* 2009]. Also in that study, an improvement of several indicators of disease was observed.

Good treatment results using antifungal medication have now been shown in three intervention studies. This suggests that antifungal medication could be a suitable alternative to corticosteroid treatment. More experience must, however, be gained regarding the long-term outcome, the clinical characteristics of those who do not respond (four out of 22 in this study), and the risk of recurrence. On the other hand, the present results are sufficient to suggest that antifungal medication should be considered in cases with corticosteroid-resistant disease. Another indication for antifungal treatment is the presence of high levels of fungal biomass in the home or workplaces. In such cases, the medical treatment should be accompanied by environmental measures and remediation actions to avoid recurrence of the disease after the termination of treatment.

In conclusion, the results demonstrate that antifungal medication could be an alternative to treatment with corticosteroids in sarcoidosis. In future studies there is a need to follow up the long-term outcome and to investigate the clinical criteria of the few patients not responding to antifungal treatment.

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### Conflict of interest statement

The authors declare that there is no conflict of interest.

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